

**CLINICOPATHOLOGIC STUDIES OF
OCCULT CARCINOMA AND OXYPHIL TUMORS OF THE THYROID
IN A DEFINED POPULATION**

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Malmö 1985

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Title and subtitle Clinicopathologic studies of occult carcinoma and oxyphil tumors of the thyroid in a defined population			
Abstract Small so called "occult" papillary carcinoma of the thyroid was found to occur in at least 6% of the population in Malmö. The frequency was of the same magnitude in men and women, and it remained much the same in the ages from the second decade. The situation in childhood is still unknown. Considering the very low rate of mortality from papillary thyroid carcinoma in the population studied, it is evident that the risk of developing fatal growth from a given occult tumor is exceedingly small. The risk is so remote that it appears justifiable to refrain from any further measures when a small papillary carcinoma is found incidentally at partial thyroidectomy, even though multiple and bilateral tumor foci as well as occult lymph node metastases are frequently present. Judging from the demographically well-defined population of Malmö, the majority of oxyphil thyroid neoplasms show a benign behaviour and can be adequately treated by hemithyroidectomy. The histopathologic picture correlates well to the clinical behaviour, and total thyroidectomy can be reserved for cases showing signs of malignancy such as metastases, blood vessel invasion or capsular penetration. Morphometric estimation of nuclear size and/or degree of anisokaryosis was found to be of no practical value for distinction between benign and malignant thyroid neoplasms of oxyphil type. The diagnostic value of microspectrophotometric nuclear DNA quantification in this respect appeared to be limited as well. Aneuploidy seems to indicate a high probability of invasive growth, but no DNA pattern permits exclusion of malignancy.			
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To my parents and my family

The present dissertation is, with addition of some hitherto unpublished results, based on the following papers:

I OCCULT THYROID CARCINOMA AT AUTOPSY IN MALMÖ; SWEDEN

Lennart Bondeson & Otto Ljungberg

Cancer 1981;47:319-323.

II OCCULT PAPILLARY THYROID CARCINOMA IN THE YOUNG AND THE AGED

Lennart Bondeson & Otto Ljungberg

Cancer 1984;53:1790-1792.

III OXYPHIL TUMORS OF THE THYROID. FOLLOW-UP OF 42 SURGICAL CASES

Lennart Bondeson, Anne-Greth Bondeson, Otto Ljungberg & Sten Tibblin

Ann Surg 1981;194:677-680.

IV MORPHOMETRIC STUDIES ON NUCLEI IN SMEARS OF FINE NEEDLE ASPIRATES FROM OXYPHILIC TUMORS OF THE THYROID

Lennart Bondeson, Anne-Greth Bondeson, Karin Lindholm, Otto Ljungberg & Sten Tibblin

Acta Cytol 1983;27:437-440.

V NUCLEAR DNA CONTENT AND BEHAVIOUR OF OXYPHIL THYROID TUMORS

Lennart Bondeson, Edward Azavedo, Anne-Greth Bondeson, Torbjörn Caspersson & Otto Ljungberg

Submitted for publication.

These papers will be referred to in the text by their Roman numerals.

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INTRODUCTION

Considering the rarity of clinically evident malignant thyroid tumors, small so called "occult" papillary carcinomas are remarkably common findings in histopathologic practice. Although this type of lesion has been well known morphologically for many years (1), knowledge about its natural history still was very incomplete when the present study was initiated in 1978. This lack of knowledge was reflected in controversy concerning the management of such tumors.

Another controversy in thyroid surgery at that time was the treatment of patients with neoplasms of oxyphil type (also called Hürthle cell tumors, Askanazy cell tumors and oncocytomas). The biological behaviour of such tumors was a matter of dispute, and opinion was also divided regarding the possibility to distinguish between benign and malignant lesions cytologically. The very divergent opinions on biological behaviour expressed by different investigators - from highly malignant (2) to mostly benign (3) - were difficult to evaluate because of lack of demographically defined study populations, and the contradictory views of different investigators regarding cytologic malignancy grading (4,5) were difficult to evaluate since they were based on subjective interpretations only.

The present study was undertaken with the following objectives:

- To estimate the prevalence, and evaluate the natural history of occult papillary carcinoma of the thyroid in a population with known incidence and mortality rates of clinical thyroid carcinoma (papers I and II).
- To evaluate the biological behaviour of oxyphil thyroid neoplasms in a demographically well-defined population (paper III).
- To evaluate the usefulness of morphometry and DNA analysis for distinction between clinically benign and malignant oxyphil thyroid tumors by objective assessment of certain cellular characteristics (papers IV and V).

MATERIAL AND METHODS

The population studied was limited to Malmö, which is an industrial coastal city with about a quarter of a million inhabitants. All surgical and radiologic treatment of thyroid disorders is performed at a single general hospital, which is served by one department of pathology. No patients are referred to other hospitals for such treatment, and the inhabitants of Malmö cannot go elsewhere in Sweden to get it on a private basis.

Autopsy was performed in 85% of all who died in Malmö during the years of interest in papers I and III. One fifth of the autopsies were medicolegal. Complete clinical autopsies were performed in 95% of all patients who died in hospital.

Prevalence and natural history of occult papillary thyroid carcinoma (I, II)

A total of 788 thyroid glands were examined. Study I is based on 500 glands representing a consecutive clinical autopsy material. Additional glands were collected with regard to special age groups for study II, which comprises 212 glands from people more than 80 years old, and 218 glands from people 50 years of age and younger. The latter group includes 116 glands obtained from medicolegal autopsies.

After fixation the glands were cut at 1-2 mm intervals, and visible lesions were selected for microscopic examination. From macroscopically normal glands at least one section from each lobe was prepared. A total of 5066 tissue blocks were examined microscopically.

Biological behaviour of oxyphil thyroid neoplasms (III)

The histopathologic records of 2002 surgical specimens registered as any kind of thyroid adenoma or carcinoma during the 19-year-period 1960-1978 were reviewed (the number of cases is influenced by the fact that multinodular colloid goiters were coded as "adenoma" until 1970, and it also includes patients referred to Malmö from other hospitals). Only tumors from patients living in Malmö at the time of their thyroid operations were investigated further. The microscopic slides were re-examined in all cases with descriptions indicating that an adenoma was composed of "large eosinophil and granular cells", "oxyphil cells", "Askanazy cells", "Hürthle cells" or "oncocytes". All cases coded as carcinomas were re-examined microscopically irrespective of the cellular description. In patients with concomitant multinodular colloid goiter or chronic thyroiditis, an oxyphil tumor was considered to be a neoplasm - and not merely a hyperplastic

nodule - only if it had a distinct capsule. Only tumors composed exclusively of oxyphil cells (Figure 1) in available sections were included in the study.

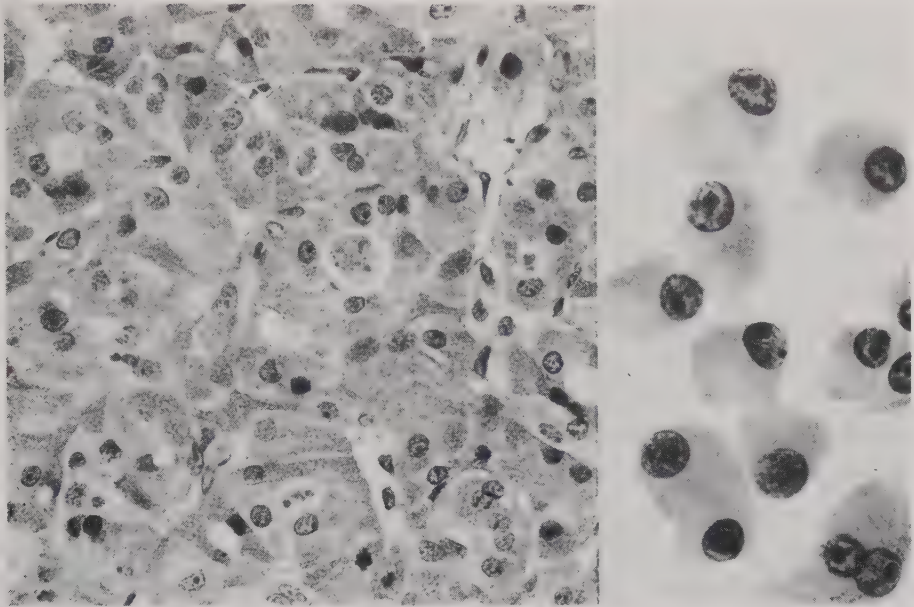


Figure 1. Typical oxyphil cells showing abundant cytoplasm with a marked granularity, which depends on abnormal accumulation of mitochondria. The cause of this cell change is unknown. It is seen in non-neoplastic as well as neoplastic thyroid lesions, and a corresponding picture occurs in many other endocrine as well as exocrine glands. The often used designation "Hürthle cells" is inappropriate. The cells observed by Hürthle in the thyroid of dogs were C-cells, and Askanazy was in 1898 the first who called attention to the cells under discussion.

A total of 42 cases of neoplastic oxyphil tumors were found. All patients still being alive in 1980 were invited to and participated in a follow-up, which comprised general physical examination including palpation of the neck, and determination of serum thyroglobulin and alkaline phosphatase levels as a screening for possible metastases. The patients have since been followed continuously by use of the population register of Malmö, and the files at the Departments of Surgery, Oncology and Pathology.

Morphometry in malignancy grading of oxyphil thyroid neoplasms (IV)

Morphometry was applied to smears of fine needle aspirates from 26 oxyphil thyroid neoplasms by use of a Leitz ASM image analysis system. All smears

were air-dried and stained according to May-Grünwald-Giemsa. In each of them the mean and standard deviation of the projected nuclear area was calculated by measurement of 200 tumor cell nuclei.

Only tumors composed exclusively of oxyphil cells in available histologic sections were included in the study. Thirteen tumors were classified as malignant; nine of these showed capsular penetration and/or blood vessel invasion as the only signs of malignancy, whereas the remaining four in addition had histologically verified metastases. Thirteen tumors were interpreted as benign on the basis of the histologic picture as well as clinical follow-up. Data on the latter 13 patients are presented in study III.

DNA analysis in malignancy grading of oxyphil thyroid neoplasms (V)

Nuclear DNA measurements were made on archival smears of fine needle aspirates from 23 oxyphil thyroid neoplasms. All smears had originally been air-dried and stained according to May-Grünwald-Giemsa. After destaining in methanol and fixation in formalin the smears were Feulgen stained. DNA quantification was made with a rapid scanning and integrating microspectrophotometer. In each specimen 100 tumor cell nuclei were measured. Leucocytes served as internal normal controls.

All tumors examined came from patients in study III. Nine tumors were classified as malignant; five of these showed capsular penetration and/or blood vessel invasion as the only signs of malignancy, whereas the remaining four in addition had histologically verified metastases and were the cause of death. Fourteen tumors were interpreted as benign on the basis of the histologic picture as well as the clinical course with follow-up from seven to 18 years.

RESULTS AND COMMENTS

Prevalence of occult carcinoma and other thyroid lesions in a general autopsy material (1)

Papillary carcinoma

Papillary thyroid carcinoma was detected in 6% of the 500 patients examined. All tumors were smaller than 1.5 cm and clinically unsuspected, thus being by definition so called "occult" carcinomas. The size limit of "occult" in this context is a matter of dispute with advocates of a maximum at only 1 or even 0.5 cm (6) depending on the fact that larger tumors can be detected by physical or scintigraphic examination, while the original definition of Woolner (7) used here is based on its prognostic significance.

Lesions with a distinct element of so called "ground-glass nuclei" (Figure 2) as well as infiltrating growth were accepted as papillary carcinomas even if papilla formation was absent. Lesions without infiltration, accepted as papillary carcinomas, showed papilla formation as well as ground-glass nuclei. Papillary lesions without ground-glass nuclei were considered malignant only if infiltrating growth was present.

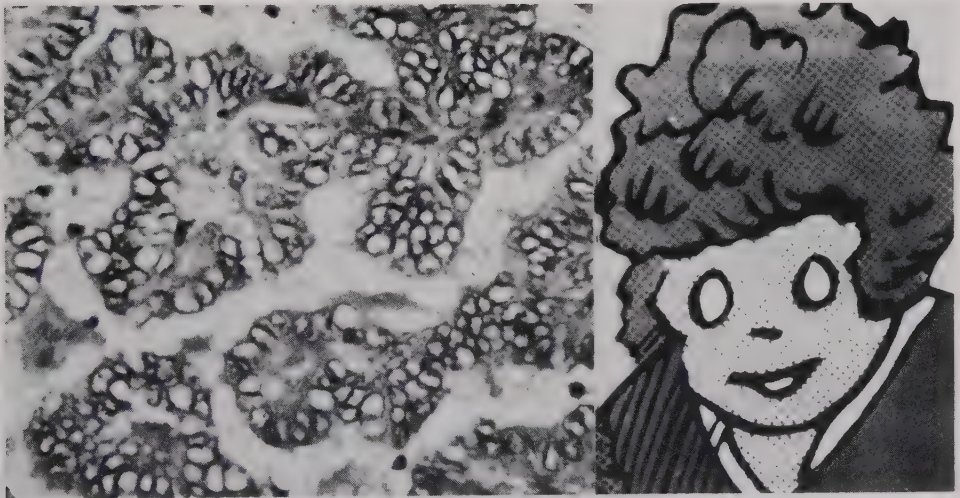


Figure 2. Typical "ground-glass nuclei" in papillary thyroid carcinoma. The sometimes used designation "Orphan Annie eye nuclei" alludes to the empty look of the little comic strip figure who also has become a musical and movie star.

The rate of occult carcinoma found is no doubt an underestimation of the true frequency since the smallest ones - being less than 1 mm and invisible for the naked eye - to a large extent escape detection with the method used here. The importance of methodologic factors in this context is well illustrated in a previous study from Japan (8). The frequency raised with some 50% when all slices of thyroid tissue was prepared for microscopy irrespective if visible lesions were present or not, and even then less than 1/30 of the tissue was properly examined. To find the smallest identifiable forms of occult carcinoma each gland should have to be serially sectioned at less than 0.1 mm intervals. However, such a meticulous work would only confirm what the less sensitive method already has shown - namely that occult papillary carcinoma is a common thyroid lesion.

The prevalence of 6% papillary carcinoma recorded in this material is close to the 5% recorded in a subsequent study from another Swedish region (9), and it is of the same magnitude as the rates of 4 - 7 % observed in other European (10,11) and American (12) populations examined by comparable techniques in recent years. It is, however, far below the more than three times higher frequencies reported from Japanese populations living not only within but also outside Japan (8,13). Since methodologic aspects and interpretation of diagnostic criteria are of great importance for the results in this context, it should be emphasized that such pronounced geographic differences in the frequency of occult papillary carcinoma have been confirmed in comparative studies performed by one and the same group of investigators (8,12,13). The reason(s) for these differences are still unknown. Genetic as well as environmental and social-cultural factors or a combination thereof may be implicated.

Multiple tumor foci were found in 19%, and bilateral growth in 16% of the papillary carcinomas. Even higher rates of multifocal distribution of occult papillary carcinoma - up to almost one third of the cases - have been reported from other materials of this kind (10,12).

Evaluation of possible regional lymph node metastases from occult papillary carcinoma was beyond the scope of the present study. This aspect has been systematically investigated in a Japanese autopsy material, which showed a rate of 16% in 128 such tumors (14). Most of these metastases were also occult in that they were not detectable clinically.

No distant metastases from occult papillary carcinoma were found, which is in accordance with the general experience that this is an exceedingly rare event. During the last 25 years only one such case has been recorded in the quarter of a million population of Malmö. That patient is alive 11 years after treatment for a histologically verified vertebral metastasis from a primary tumor measuring 8 mm.

Follicular neoplasia

Follicular neoplasia was diagnosed in 29 patients (6%) with the exception of possible macrofollicular "colloid" adenomas, which have been excluded here since it may be difficult or even impossible to distinguish that type of tumor from non-neoplastic nodular colloid goiter.

Seven follicular tumors were considered malignant because of blood vessel invasion and/or capsular penetration. Four of these measured less than 1 cm. None showed metastases. This relatively high frequency of "histologic" follicular carcinoma compared to other similar studies may simply be a reflection of the fact that the capsular region was extensively examined by serial sectioning in many of the follicular tumors.

The prevalence of follicular neoplasia (with the limitation mentioned above) was the same in women (13/221) and men (16/279), which is surprising considering the marked female predominance of such tumors in clinical practice. There was, however, a distinct difference as regards tumor size. Half of the female tumors measured more than 1 cm, and were thereby clinically detectable, while four fifths of the male tumors were smaller.

People with follicular neoplasia showed the same rate of papillary carcinoma as the population in general.

Medullary carcinoma

Suspected C-cell proliferations were tested for argyrophilia with Grimelius' method, and lesions containing argyrophil cells were further investigated for calcitonin immunoreactivity. Four cases of medullary carcinoma were detected in study I, and three more were encountered in the additional material collected for study II. Thus, a total of seven medullary carcinomas occurred in 788 patients (1%). All were solitary lesions. The largest one measured 1.5 cm, while the others were smaller than 0.5 cm. None showed metastases.

Compared to similar studies from other countries, this frequency of medullary carcinoma is very high. It is not possible to determine if the difference is a true geographic one, or merely a reflection of the special methods used here, which allowed diagnosis even in tumors with no detectable stromal amyloid. There was no indication that any of these tumors was part of a familial multiple endocrine neoplasia syndrome.

Non-neoplastic goiter

The median weight of the thyroid glands was 18 g. The rate of goiter in the material as a whole was 9% (Figure 3) if a weight of 35 g is considered the upper limit of the normal range as has previously been suggested (13).

There was no significant difference in the rate of papillary carcinoma in goitrous patients compared to patients with glands of normal weight. This finding is in agreement with the experience from other similar studies (10), including those of goitrous populations in Sweden (9) and Poland (13).

The presence of multinodular changes as such - irrespective of glandular weight - was not associated with any difference in the rate of papillary carcinoma as compared to non-nodular glands. Clinically detectable "colloid nodules" occurred in 27% of all female and 7% of all male glands (Figure 4), if a nodule larger than 1 cm is considered palpable. This definition seems relevant since the rate in middle-aged women of this material (13%) is in good agreement with the prevalence of palpable thyroid lesions recorded (11%) in the same population group in a recent health screening study performed in Malmö (15).

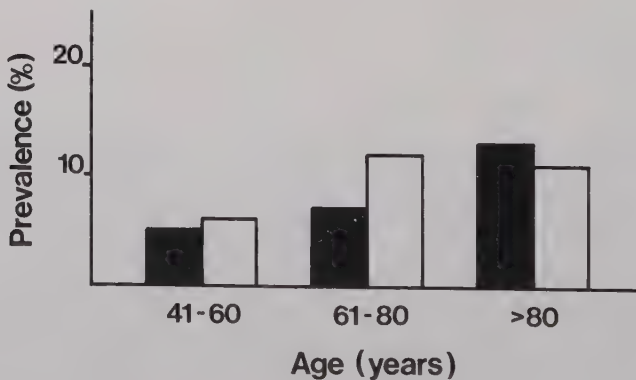


Figure 3. Frequency of diffuse and nodular goiter (thyroid weight over 35 g) by age and sex (males black).

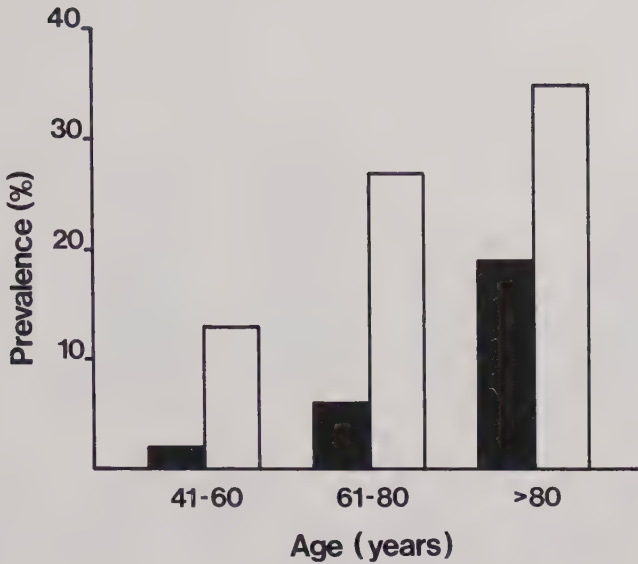


Figure 4. Frequency of multinodular glands of all weights (with at least one nodule larger than 1 cm) by age and sex (males black).

Thyroiditis

A chronic nonspecific inflammation with varying degree of multifocal or diffuse infiltration of lymphatic cells occurred in 13% of the female and 5% of the male glands (Figure 5). There was no significant difference in the rate of papillary carcinoma in patients with chronic thyroiditis compared to other patients.

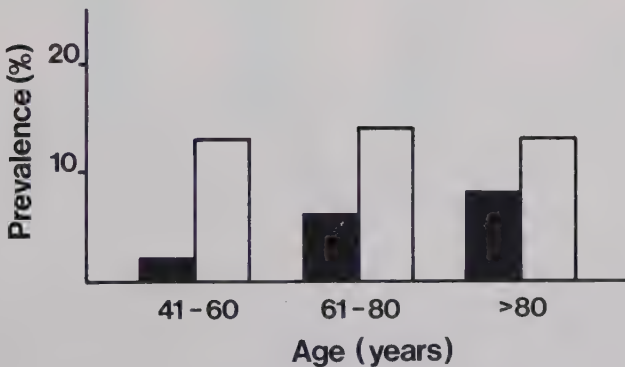


Figure 5. Frequency of chronic thyroiditis by age and sex (males black).

The thyroid in patients with malignant neoplasms of other sites

Malignant tumors in sites other than the thyroid - present at autopsy as well as previously cured (excluding in situ lesions) - were recorded in 40% of the patients with primary thyroid carcinoma. The corresponding rate in patients without thyroid carcinoma was 51%. Thus, no association between carcinoma of the thyroid and occurrence of other malignancies was found, and this material does not provide any support for older reports suggesting an increased incidence of other malignant neoplasms in patients with thyroid carcinoma (16).

Thyroid metastases were detected in 16% of all patients with metastasizing malignant solid neoplasms. Among the many kinds of primary tumors represented, the most common were carcinoma of the breast and kidney. The metastatic lesions were often multifocal and bilateral (one half and one third of the cases respectively). The majority (75%) were smaller than 1 cm and clinically unsuspected. Usually thyroid metastases were part of widespread disease (on an average five other organs involved). The only exception was a case of renal carcinoma, removed 14 years earlier, with a solitary metastasis localized to the thyroid at autopsy.

Natural history of occult papillary carcinoma of the thyroid (I, II)

Prevalence by age

Occult papillary carcinoma occurred in much the same frequency in the ages from the second decade (Figure 6). The youngest person with the lesion in question was 17 years of age. The number of teenagers was too small to warrant a reliable estimation of the frequency in this age group, but the finding of two carcinomas among a total of eight youngsters less than 20 years of age deserves notice.

It remains to be clarified whether most of these tumors arise early in life and then remain dormant with minimal growth, or whether new tumors steadily arise at all ages, some of which regress and vanish spontaneously, thus keeping the frequency at a relatively constant level.

Prevalence by sex

In contrast to a marked female preponderance of clinical papillary thyroid carcinoma, the frequency of occult tumors was of the same magnitude in women (8%) and men (6%) in the population studied (Figure 7).

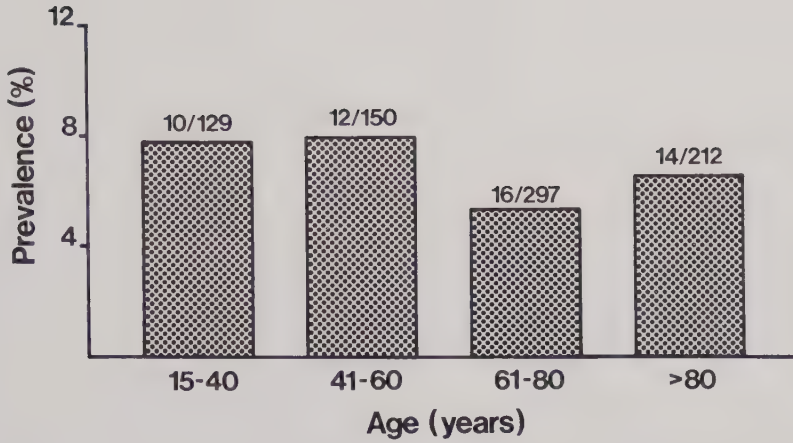


Figure 6. Frequency of occult papillary thyroid carcinoma by age.

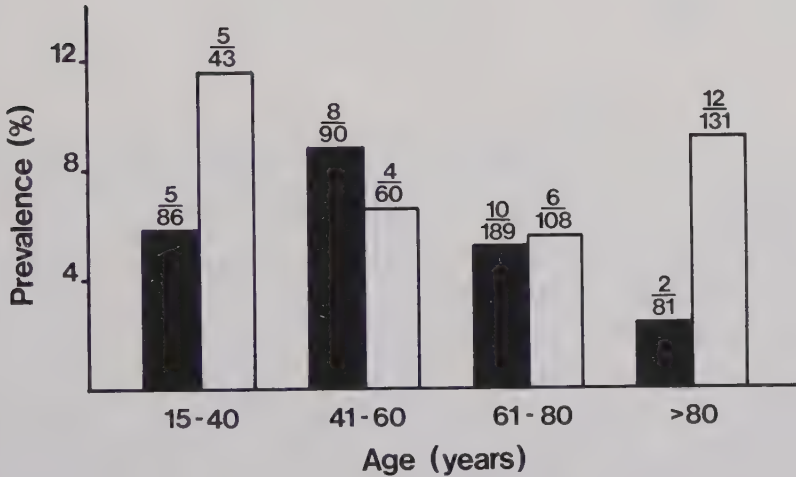


Figure 7. Frequency of occult papillary thyroid carcinoma by sex in different age groups (males black). The female predominance in the youngest and oldest groups is not statistically significant.

Size by sex and age

The tumors of the women were on an average larger (4.3 mm) than those of the men (2.6 mm), which is in line with the experience of others (8). Six out of eight tumors measuring more than 5 mm occurred in women. Furthermore, there was a slight increase of the average tumor size with advancing age in the women, while the male tumors rather showed a tendency to become smaller (Figure 8). This is in line with the assumption that female tumors are subjected to some growth promoting factor(s) - different from the ones that cause induction of the tumor - explaining the marked female predominance of clinically evident papillary thyroid carcinoma in spite of the equal sex ratio of occult tumors (17). Alternatively male tumors are influenced by some growth inhibiting factor(s).

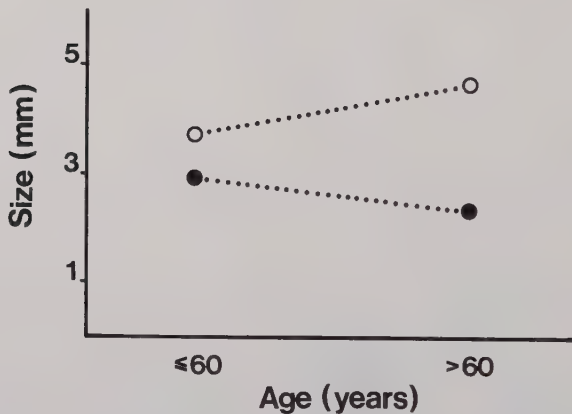


Figure 8. Means of tumor size by age and sex (males black).

The differences in size was of the same magnitude whether calculated from the largest width of the tumors, or from their largest cut surface area which better reflects the tumor volume and growth potential (area measured by a Leitz ASM image analysis system). This deserves notice since the tumors varied much in shape, and a spiculated tumor with long "arms" and a tiny "body" could have the same maximum diameter as a spherical tumor with much larger tumor cell mass.

Morphologic aspects

A markedly infiltrating growth pattern was the most common one, but a substantial number of tumors were well circumscribed with pushing instead of infiltrating margins. There was no appreciable difference in this respect regarding age or sex.

Lymphoid infiltration of the tumor was uncommon on the whole, and occurred with one exception only in patients with chronic thyroiditis.

The amount of fibrous tissue in the tumors varied considerably without any appreciable correlation to either age or sex. It was not possible to determine if tumor sclerosis reflected start of tumor growth in a scar, tumor involution, or merely a stroma reaction secondary to tumor growth.

Comparisons with other populations

Only two previous autopsy studies of thyroid carcinoma, from Japan and Portugal, have included a substantial number of cases in the both extremes of age (8,10). While the Japanese material did not show any increased frequency of occult papillary carcinoma with advancing age after a peak in the forties, a somewhat linear increase with age was found in Portugal. This difference might be spurious and explained by a less sensitive method of examination used in the Portuguese study, causing an overrepresentation of tumors in elderly women since their tumors are generally larger and thereby easier to find.

Clinical significance of occult papillary thyroid carcinoma

The occurrence of clinically evident thyroid carcinoma in the population of Malmö is well known from other studies (18,19). During the 18-year-period 1960 - 1977, 53 patients were treated for symptomatic papillary carcinoma, corresponding to an annual incidence of three patients among a quarter of a million inhabitants. During the same period of time, 11 patients died from growth of papillary carcinoma (seven cases), or anaplastic carcinoma with a differentiated papillary component (four cases), giving a mortality rate of one patient in about two years. These figures are to be compared with the constant presence of at least 15000 occult papillary carcinomas in the same population. Although all clinically evident and fatal tumors were at one time "occult", this comparison gives some idea of the exceedingly small risk that a given occult tumor will have such a course.

Biological behaviour of oxyphil neoplasms of the thyroid (III)

Patient characteristics

The female to male ratio was 4:1 among the 42 patients operated on for oxyphil tumors. The youngest patient was only 16 years of age, but most tumors were found in middle-aged and older people. The age and sex distribution of the patients in the present material is in agreement with the general experience with this kind of tumor.

Outcome of surgical treatment

Total thyroidectomy was performed in six patients, while 36 had less extensive operations (mostly lobectomies).

No patient has been lost to follow-up. Thirty-two patients are alive at present. All living patients now have an observation time of at least seven years (mean 12 years), and none of them has presented any evidence of local recurrence or metastases of their thyroid tumors. Eight patients have died from other diseases. None of these patients showed any remnants of the tumor in question at autopsy.

Two patients have died of metastases from their thyroid tumors (both initially treated with total thyroidectomy). One of them had cervical lymph node metastases at the time of operation and developed liver metastases three years later. In the other patient lung metastases and recurrent growth in the neck occurred seven years after thyroidectomy.

A most often benign behaviour of oxyphil thyroid neoplasms has been reported in several subsequent studies from other places (20-23). However, the results are the exact opposite to the experience at the University of Michigan in Ann Arbor (2,24) where all such tumors are considered potentially malignant with a dismal prognosis. Among several possibly contributing factors (25), the probably most important reason for this discrepancy is a selection of prognostically bad cases in Ann Arbor, being referred to the surgical expertise there from many places in the United States because of apparent or suspected malignant disease. The same phenomenon might explain the predominance of malignant cases reported from the University of Zürich (26). Proper judgement of the relative frequencies of clinically benign and malignant disease in this context requires a defined study population in which all treated thyroid tumors are known. So far the present material is the only one known to fulfil this prerequisite.

Two other factors being important for the validity of the conclusions drawn from this material are the length of follow-up, and the diagnostic accuracy regarding separation of true neoplasms from merely hyperplastic nodules of oxyphil cells.

In studies indicating a predominantly malignant behaviour (2,24,26), the mean interval from initial treatment to detection of recurrent disease has been between 3.5 and 4.1 years. Thus, the observation time of the present material (minimum seven, mean 12 years) appears to be sufficiently long to permit conclusions regarding prognosis in general.

Since the distinction between a true adenoma and a non-neoplastic nodule of oxyphil cells occurring in association with multinodular colloid goiter and chronic thyroiditis always can be questioned regardless of the criteria used, it shall be underlined that only three out of 34 tumors here classified as adenomas were associated with such disorders. Thus, the conclusions obtained from this material would have remained the same even if these few cases had been left out of account.

Non-surgical cases of oxyphil thyroid carcinoma

To exclude the possibility that a falsely good prognosis in this material was caused by absence of patients never operated on because of too advanced disease at the time of admission, the autopsy files of Malmö were reviewed with regard to deaths related to growth of thyroid carcinoma. During the period from which the present surgical material derives (1960 - 1978), two patients died of oxyphil thyroid carcinoma. Both had been considered inoperable and given radiotherapy only. These two patients constitute 9% of all who died of thyroid carcinoma in the population of Malmö during that period. This figure is of the same magnitude as the 7% proportion of oxyphil carcinoma among all clinical thyroid carcinoma in Malmö, which indicates that the oxyphil cell change as such is of no prognostic significance.

Histologic findings and prediction of prognosis

Eight tumors in the surgical material showed histologic signs of malignancy judging from the criteria commonly used for follicular neoplasia of the thyroid - i.e. blood vessel invasion and/or capsular penetration. The two metastasizing and fatal tumors belonged to this group. All of the histologically benign tumors have had a benign clinical course. Thus, the malignant potential of a given oxyphil thyroid neoplasm appears to be accurately reflected by its histologic picture. This finding is in opposition to the opinion in Ann Arbor (2,24), but in agreement with the experience elsewhere (20-23,26,27).

Morphometry in malignancy grading of oxyphil thyroid neoplasms (IV)

Malignant tumors were found to have a somewhat larger mean nuclear size than the benign ones when compared as groups. There was, however, an almost complete overlapping between the two tumor categories with regard to individual values. The degree of variation in nuclear size likewise showed an almost complete overlapping between benign and malignant tumors.

The values obtained were well reproducible in one and the same case, and the overlapping observed between different cases was not related to technical factors involved with the smear preparation.

It may be objected that cases with marked anisokaryosis and large mean nuclear size considered benign, could in fact have been successfully treated carcinomas despite the lack of histologic signs of malignancy in the tissue samples examined. This possibility cannot be excluded, but more important is, however, that several carcinomas - including metastasizing ones - occurred among tumors with the smallest mean nuclear size as well as the least pronounced anisokaryosis, which means that malignancy cannot be ruled out with this method.

Thus, morphometric estimation of nuclear size and/or degree of anisokaryosis is of no practical value for distinction between benign and malignant thyroid neoplasms of oxyphil cell type, in contrast to what has been reported for non-oxyphil follicular neoplasms (28).

DNA analysis in malignancy grading of oxyphil thyroid neoplasms (V)

The 23 tumors examined could be divided into three main groups with regard to DNA pattern, i.e. diploid, polyploid or aneuploid. Euploid patterns (diploid or polyploid) were encountered in 17 patients, who showed long survival after surgical treatment and most often had histologically benign tumors. However, also histologically malignant tumors occurred in these groups, and two patients with euploid tumors died of metastases. Aneuploidy, on the other hand, was frequently associated with an apparent invasive potential as illustrated by a histologically malignant growth pattern in five out of six cases. However, the aneuploid tumors showed a variable clinical course with examples of long and clinically tumor-free survival after surgery as well as rapid death from metastases.

From a practical standpoint the main contribution of microspectrophotometric DNA measurement in oxyphil thyroid tumors is a possibility to demonstrate aneuploidy by means of fine needle aspiration. As has been reported for follicular neoplasms of non-oxyphil type (29,30), this finding seems to reflect a high probability of malignancy, which may be of decisive importance in the management of patients who because of other disease represent "surgical risks".

GENERAL SUMMARY AND CONCLUSIONS

Small so called "occult" papillary carcinoma of the thyroid was found to occur in at least 6% of the population in Malmö. The frequency was of the same magnitude in men and women, and it remained much the same in the ages from the second decade. The situation in childhood is still unknown. Considering the very low rate of mortality from papillary thyroid carcinoma in the population studied, it is evident that the risk of developing fatal growth from a given occult tumor is exceedingly small. The risk is so remote that it appears justifiable to refrain from any further measures when a small papillary carcinoma is found incidentally at partial thyroidectomy, even though multiple and bilateral tumor foci as well as occult lymph node metastases are frequently present.

Judging from the demographically well-defined population of Malmö, the majority of oxyphil thyroid neoplasms show a benign behaviour and can be adequately treated by hemithyroidectomy. The histopathologic picture correlates well to the clinical behaviour, and total thyroidectomy can be reserved for cases showing signs of malignancy such as metastases, blood vessel invasion or capsular penetration.

Morphometric estimation of nuclear size and/or degree of anisokaryosis was found to be of no practical value for distinction between benign and malignant thyroid neoplasms of oxyphil type. The diagnostic value of microspectrophotometric nuclear DNA quantification in this respect appeared to be limited as well. Aneuploidy seems to indicate a high probability of invasive growth, but no DNA pattern permits exclusion of malignancy.

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PAPERS I - V

Occult Thyroid Carcinoma at Autopsy in Malmö, Sweden

LENNART BONDESON, MD, AND OTTO LJUNGBERG, MD

Thorough examination of complete thyroid glands from 500 consecutive autopsies in a Swedish population revealed 43 primary thyroid carcinomas (8.6%). Thirty-two were papillary, seven follicular, and four medullary. All but three follicular carcinomas were less than 1 cm and clinically unsuspected. The prevalence of occult papillary carcinoma did not differ significantly between men and women and there was no definite increase in frequency with age in the age groups studied (40–90 years).

Cancer 47:319–323, 1981.

ALTHOUGH CLINICALLY EVIDENT thyroid carcinoma is rare, small "occult" thyroid carcinomas appear to be surprisingly frequent in thoroughly examined autopsy series. Comparable studies are still few in number but it is apparent from these that there are marked geographical differences in the occurrence of such tumors. The situation in Sweden has hitherto not been elucidated, so this study was undertaken to determine the prevalence of thyroid carcinoma with special reference to "occult" tumors in a Swedish population.

Materials and Methods

Thyroid glands were obtained from 510 consecutive autopsies performed at Malmö General Hospital during five months in 1979. Ten cases that had been subjected to thyroid surgery were excluded because resected parts of these glands were lost from the study. Furthermore, the diagnoses were not available in several instances. The distribution of the examined 500 glands by sex and age appears in Table 1.

The glands were weighed and fixed in 10% formalin. Enlarged glands were partially sectioned to obtain a good state of preservation, otherwise the glands were intact when fixed. After fixation the glands were cut at 1–2 mm intervals with a scalpel. Visible lesions were recorded in drawings and selected for microscopic examination. At least one histologic section was prepared from each lobe of macroscopically normal glands. Hematoxylin-erythrosin was used for routine staining. Suspected C-cell proliferations were first

tested for argyrophilia with Grimelius silver nitrate procedure.⁵ Lesions with argyrophil cells were further investigated for the presence of calcitonin by an indirect immunofluorescence technique⁴ using paraffin sections which were deparaffinized and refixed in Bouin's solution for three hours. Calcitonin antiserum raised in rabbit (Batch AS292/5 used in dilution 1:20) was kindly provided by Prof. I. MacIntyre, Endocrine Unit, Postgraduate Medical School, London. C-cell lesions were also examined for amyloid with alkaline Congo red.¹⁰

The slides were examined continuously as the study progressed. When equivocal lesions occurred, additional sections were prepared. After the material as a whole was complete all slides were reviewed and the diagnoses finally determined. The criteria used for classification of the tumors corresponded to those advocated by Hazard.⁶

Results

A total of 43 primary thyroid carcinomas (8.6%) was detected. Thirty-two were papillary, seven follicular, and four medullary. The distribution of the tumors by sex, age, histologic type, and size is given in Tables 2 and 3. None of these carcinomas was the cause of death.

All but two tumors classified as papillary carcinomas showed papilla formation. The two exceptions were

TABLE 1. Age and Sex Distribution of 500 Autopsies

Age groups (yrs)	Women	Men
0–20	—	3
21–40	6	6
41–60	31	44
61–80	108	189
>80	76	37
TOTAL	221	279

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TABLE 2. Age and Sex Distribution of 43 Thyroid Carcinomas

Type of carcinoma	Sex	No. of carcinomas		
		41-60 years	61-80 years	>80 years
Papillary	F	3	6	9
	M	3	10	1
Follicular	F	1	1	2
	M	—	3	—
Medullary	F	1	1	1
	M	—	1	—

TABLE 3. Tumor Size in 43 Thyroid Carcinomas

Type of carcinoma	Sex	No. of carcinomas		
		<3 mm	3-10 mm	>10 mm
Papillary	F	6	12	—
	M	8	6	—
Follicular	F	1	1	2
	M	—	2	1
Medullary	F	2	1	—
	M	1	—	—

examples of "nonencapsulated sclerosing microcarcinomas"¹⁶ (Fig. 1) with a stellate scar and infiltrating follicles lined by cells demonstrating the vesicular

"ground glass" nuclei characteristic of papillary carcinoma. Such nuclei were absent in some papillary carcinomas but all, however, showed infiltrative growth leaving no doubt about their malignancy. Tumors without obvious infiltration, accepted as papillary carcinomas, invariably showed papilla formation as well as typical "ground glass" nuclei. In six cases (20%) of papillary carcinoma the thyroid contained multiple foci of tumor. In five of these both lobes were involved. One papillary carcinoma metastasized to a cervical lymph node.

The tumors classified as follicular carcinomas were devoid of papillae and "ground glass" nuclei. In six of the seven cases of this type invasion of blood vessels or capsular penetration was evident. In the remaining case the diagnosis was based on focal infiltration into surrounding parenchyma (Fig. 2). This lesion showed a marked sclerosis which seemed to indicate papillary carcinoma rather than follicular. However, cellular features were not typical of papillary carcinoma, and the tumor was examined in its entirety without discovering papilla formation.

In three of four tumors classified as medullary carcinomas calcitonin was demonstrated in tumor cells by immunofluorescence. Shortage of tissue prevented this trial in the fourth case. However, argyrophilia of tumor cells in addition to the histologic architecture of this lesion (Fig. 3) seemed to justify the diagnosis. No

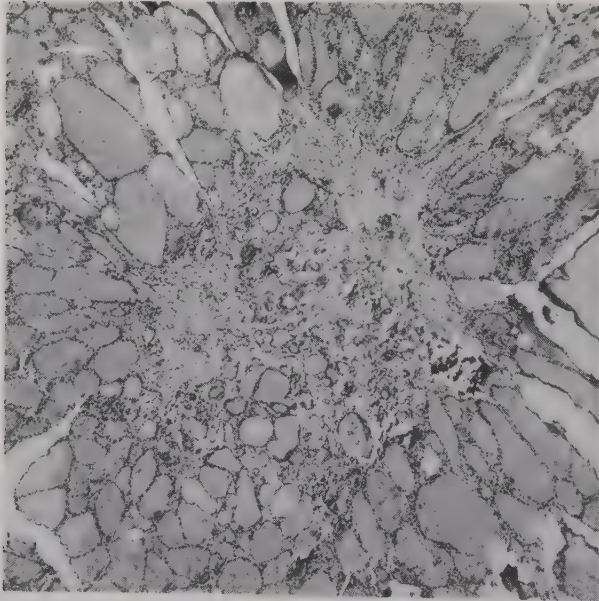


FIG. 1. Nonencapsulated sclerosing papillary carcinoma (H & E, $\times 40$).

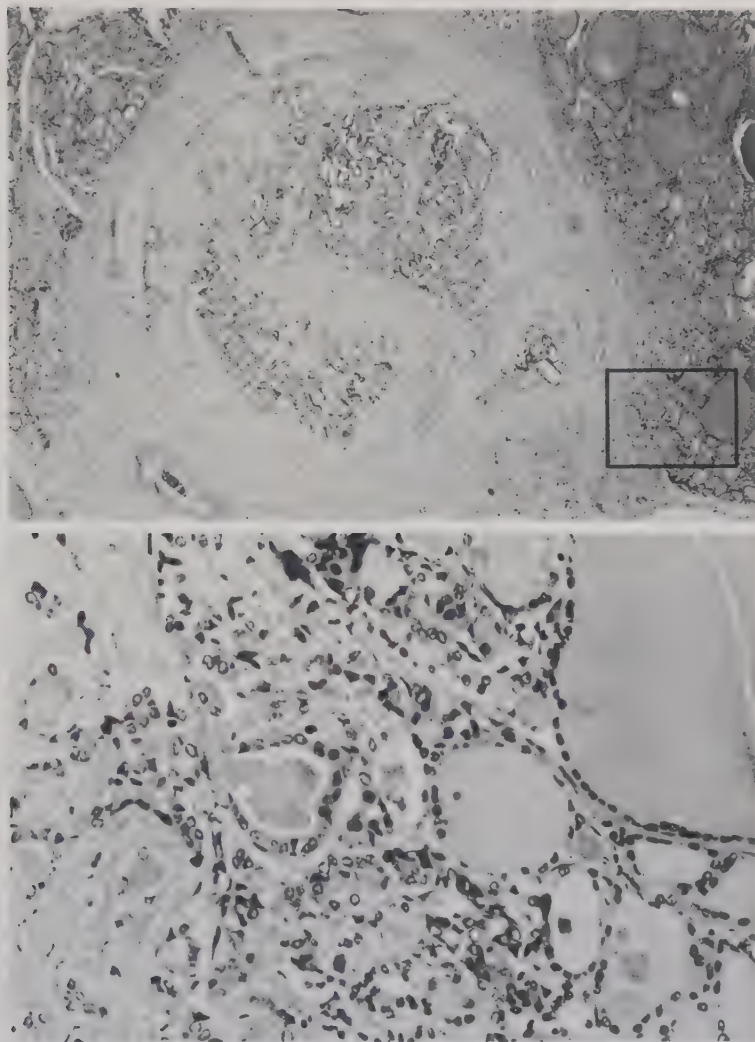


FIG. 2. (Top): Follicular carcinoma of unusual appearance with marked sclerosis. (H & E, $\times 40$) (Bottom): Area within box is shown in detail. Note focal infiltration into surrounding parenchyma (H & E, $\times 320$).

amyloid deposits were detected in any of these tumors. In one case the medullary carcinoma occurred together with a papillary carcinoma. In another case a papillary carcinoma was accompanied by an area with marked interfollicular proliferations of cells containing immunoreactive calcitonin. This C-cell lesion (Fig. 4) did not show any distinct nodule formation or definite parenchymal destruction, which was required for a diagnosis of medullary carcinoma. It was interpreted therefore as a focal C-cell hyperplasia.⁷

The weight of the glands varied from 3–240 g with a median of 18 g. If a weight of 35 g is considered the upper limit of the normal range, as has previously been suggested,³ the rate of goiter was 11% in women and 7% in men, one-fourth being diffuse and the rest multinodular. Five papillary carcinomas were found in multinodular goiters.

A chronic nonspecific inflammation with varying degree of multifocal or diffuse infiltration of lymphocytes and plasma cells was found in 13% of the female

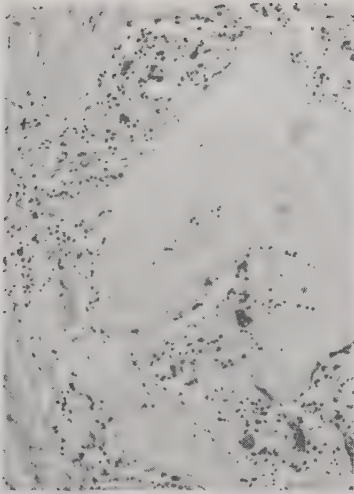


FIG. 3. Medullary carcinoma with irregular nests of small dark cells in abundant hyaline stroma (H & E, $\times 125$).

and 5% of the male glands. Six papillary carcinomas and one follicular carcinoma occurred together with chronic thyroiditis.

Discussion

With the exception of three follicular tumors, which measured between 2.5 and 4 cm, all carcinomas in this

study were less than 1 cm in largest dimension and clinically unsuspected, thus being by definition so-called occult carcinomas.¹⁴ The smallest tumors found were six papillary carcinomas sized 1 mm or slightly less. The female patients' tumors were on an average somewhat larger than those of the male patients.

The observed 8% prevalence of occult thyroid carcinoma contrasts with the low annual incidence of thyroid carcinoma reported to the Cancer Registry in Sweden. The latest figures available, from 1973, for age standardized incidence rate were 4.9 (women) and 2.1 (men) per 100,000 of the mean population.⁸

In contrast to the marked female preponderance for clinical thyroid carcinoma, the prevalence of occult carcinoma found in the present series did not differ significantly between men and women. Moreover, there was no significant change in frequency with age. It should be noted, however, that virtually all patients were over 40 years of age.

Similar previous investigations from other parts of the world performed in recent years are summarized in Table 4. In comparing these studies, it must be emphasized that the figures obtained are highly dependent not only on diagnostic criteria and their subjective application but also on the methods used because the lesions in question may be extremely small. Furthermore, it should be observed that in previous series papillary carcinomas make up the vast majority of recorded thyroid carcinomas, but there are few nonpapillary carcinomas. In the large study from Hiroshima and Nagasaki¹¹ of 2426 glands, only eight follicular carcinomas and one medullary carcinoma were found. The extensive investigation by

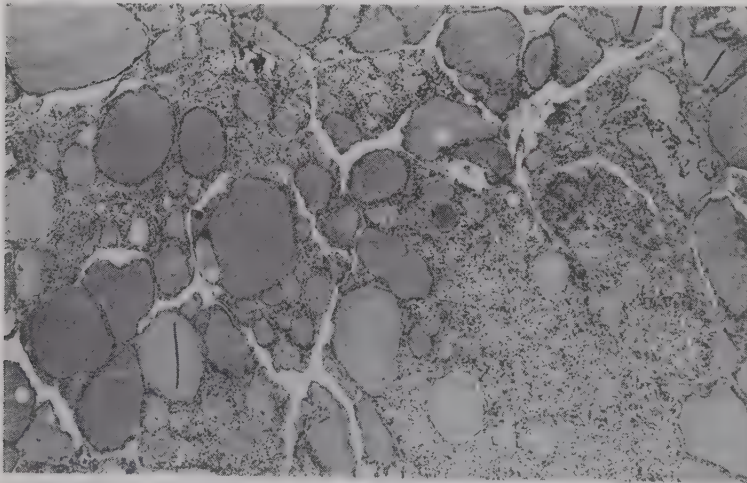


FIG. 4. Marked focal C-cell hyperplasia. Note interfollicular cell proliferations without distinct nodule formation (H & E, $\times 40$).

TABLE 4. Prevalence of Thyroid Carcinoma in Thoroughly Examined Recent Autopsy Series

Authors	Sources of material	No. carcinoma total glands	Prevalence (%)
<i>American populations</i>			
Sampson <i>et al.</i> ¹²	Olmstead County	9/157	5.7
Nishiyama <i>et al.</i> ⁹	Ann Arbor	13/100*	13.0
<i>Japanese populations</i>			
Sampson <i>et al.</i> ¹¹	Hiroshima & Nagasaki	384/2035	18.9
Sampson <i>et al.</i> ¹¹	Hiroshima & Nagasaki	111/391*	28.4
Fukunaga & Lockett ²	Hawaii	24/100*	24.0
Fukunaga & Yatani ³	Hawaii	60/248*	24.2
Fukunaga & Yatani ³	Sendai	29/102*	28.4
<i>Other populations</i>			
Fukunaga & Yatani ³	Canada	6/100*	6.0
Fukunaga & Yatani ³	Columbia	34/607*	5.6
Fukunaga & Yatani ³	Poland	10/110*	9.1
Balázs & Krasznai ¹	Hungary	9/200	4.5
Sobrinho-Simoes <i>et al.</i> ¹³	Portugal	39/600	6.5
Present series	Sweden	43/500	8.6

* Microscopic slide made from each slice of serially sectioned glands. In the other series only grossly visible lesions were examined.

Fukunaga and Yatani³ with a total of 1167 glands from different countries revealed only a single follicular carcinoma. In a study of 600 Portuguese glands¹³ only papillary carcinomas were found. Thus it appears that

follicular as well as medullary carcinomas are comparatively more frequent in this Swedish study.

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Occult Papillary Thyroid Carcinoma in the Young and the Aged

LENNART BONDESON, MD, AND OTTO LJUNGBERG, MD

Thorough examination of complete thyroid glands from the autopsies of 218 people 50 years of age or younger, and 212 persons older than 80 years of age in a Swedish population showed that occult papillary thyroid carcinoma occurred in much the same frequency (about 7%) in the ages from the second decade. The youngest person with the lesion in question was 17 years of age. The prevalence of occult papillary thyroid carcinoma did not differ significantly between men and women. The tumors of the women were on an average larger than those of the men, and the tumors in the old were on an average larger than those in the young.

Cancer 53:1790-1792, 1984.

SEVERAL AUTOPSY STUDIES from various places have confirmed a considerable prevalence of minimal so-called occult papillary carcinoma of the thyroid.¹ In Malmö we have recently estimated this prevalence to be at least 6% of the population over 50 years of age.¹ Most investigations in this context have, like ours, been performed on general autopsy material with few younger people represented. Thus, the occurrence of occult papillary thyroid carcinoma before middle age is not well evaluated. Moreover, the natural history of this lesion is incompletely known. To shed some light on these issues we have extended our previous study to include people younger than 50 years of age and over 80 years of age.

Material and Methods

In the current material we have included 142 thyroid glands from a previous study,¹ comprising 29 from people 50 years of age or younger, and 113 from people older than 80 years. In addition, we have collected consecutively another 73 thyroid glands in the former category, and 99 thyroid glands in the latter category from clinical autopsies performed at Malmö General Hospital. As regards people aged 50 years or younger, we have obtained 116 more thyroid glands from medicolegal autopsies performed in Malmö because of death by accident or suicide. In this group the glands could not be collected consecutively in order to avoid pronounced postmortem or traumatic changes, which would render an adequate examination

in this context impossible. The material comprises in all 218 thyroid glands from people 50 years of age or younger, and 212 glands from people over 80 years of age. The distribution of the glands by age and by sex appears in Table 1.

All glands in this study were complete. None of the patients had been subjected to any thyroid operation. The methods of preservation and examination of the glands conformed in every detail with the procedure previously described.¹ In short, the glands were cut manually at 1 to 2 mm intervals with a scalpel after fixation in 10% formalin. Visible lesions were selected for microscopic examination. From macroscopically normal glands at least one section from each lobe was prepared. Hematoxylin-erythrosin was used for routine staining.

Results

Primary thyroid carcinomas were detected in 19 of the 218 glands in the younger group. All but one of these tumors were classified as papillary carcinomas by the same criteria as previously outlined.¹ The remaining lesion was a solitary medullary carcinoma with calcitonin immunoreactivity and amyloid deposits demonstrated in accordance with methods previously stated.¹ The latter tumor, which measured 1.5 cm, occurred in a 43-year-old man. The 18 papillary carcinomas measured at most 10 mm in largest dimension, and they were all clinically unknown. Tumor size is given together with age and sex in each case in Table 2. Twelve of the 18 papillary carcinomas came from the medicolegal material.

Primary thyroid carcinomas were detected in 19 of the 212 glands in the old group. Fourteen of these tumors were classified as papillary carcinomas. None of them measured more than 12 mm in largest dimension, and

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TABLE 1. Age and Sex Distribution of 430 Autopsied Patients

Age groups (yr)	Women	Men
11-20	5	7
21-30	15	35
31-40	23	44
41-50	34	55
81-90	106	67
91-100	25	14

they were all clinically unknown. Tumor size is given together with age and sex in each case in Table 3. Two tumors were classified as follicular carcinomas, and the remaining three as medullary carcinomas. The latter occurred as solitary tumors and measured 2 to 4 mm. Calcitonin immunoreactivity was demonstrated in all of them, and amyloid deposits in one. All of the nonpapillary carcinomas in this group occurred in women.

Multifocal papillary carcinomas were detected in three glands in the younger group, and in two glands in the older group. In Tables 2 and 3 the measures of the largest of the multiple foci are given in each of these five cases.

Discussion

The prevalence of occult papillary thyroid carcinoma in the population of Malmö was found to be much the same (about 7%) among people younger than 50 and older than 80 years of age. In the younger group the earliest cases found occurred at the age of 17 years (Figs. 1 and 2). The finding of occult papillary thyroid carcinoma at this age is not too remarkable, *per se*, since papillary thyroid carcinoma is occasionally seen in children in sur-

TABLE 3. Age, Sex, and Tumor Size in 14 Cases of Papillary Thyroid Carcinoma From Patients Older Than 80 Years of Age

Age (yr)	Sex	Largest tumor width (mm)
81	M	3
81	F	4
82	F	4
84	F	12
84	F	4
85	M	2
86	F	3
87	F	4
88	F	5
90	F	8
90	F	3
91	F	1
92	F	4
100	F	12

Mean tumor width (mm): M = 2.5; F = 5.3; M + F = 4.9.

gical practice. Nevertheless, the finding of two such lesions among a total of eight youngsters less than 20-years-old is somewhat surprising. The number of cases in this age group is, however, too small to warrant a reliable estimation of the true frequency of occult papillary carcinomas in teenagers.

In the younger group the prevalence of occult papillary thyroid carcinoma was almost the same among men and women. In the older group there was a female predominance, but this was not statistically significant.

Histologic features, including the degree of sclerosis, of the papillary carcinomas in the younger group did not differ appreciably from those in the older one. The papillary carcinomas of the women were on an average larger than those of the men. In the older group the tumors were on an average somewhat larger than in the younger

TABLE 2. Age, Sex, and Tumor Size in 18 Cases of Papillary Thyroid Carcinoma From Patients 50 Years of Age and Younger

Age (yr)	Sex	Largest tumor width (mm)
17	M	1
17	F	2
21	F	2
29	M	2
30	F	4
32	M	2
35	F	2
38	M	0.9
40	F	7
40	M	1
43	M	1
45	F	10
45	M	5
46	M	1
47	F	1
47	M	2
50	M	3
50	M	8

Mean tumor width (mm): M = 2.4; F = 4.0; M + F = 3.1.

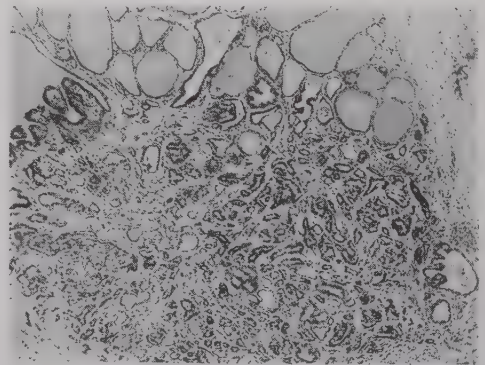


FIG. 1. Typical growth pattern and stromal sclerosis in an occult papillary thyroid carcinoma from a 17-year-old woman (H & E, original magnification $\times 61$).

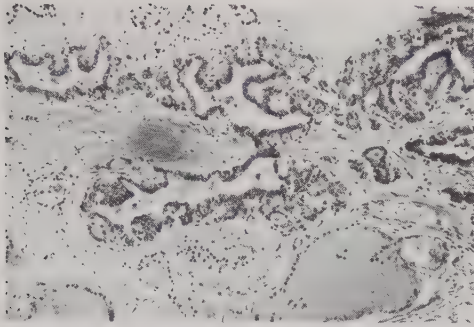


FIG. 2. One of the smallest papillary carcinomas in this series, coming from a 17-year-old man. Notice the typical cellular features with "ground glass" nuclei (H & E, original magnification $\times 153$).

one, even when considering the sex difference in the composition of the groups.

Only two previous autopsy studies of thyroid carcinoma, from Japan and Portugal, have included a substantial number of cases in both of the age groups under discussion (Tables 4 and 5). The conclusions of these studies differ with respect to the natural history of occult papillary thyroid carcinoma. In the Japanese study by Sampson and coworkers,² there was no increase in prevalence with age after a peak in the ages around 40 years. Instead, a tendency for decrease in prevalence with advancing age was found. On the contrary, Sobrinho-Simoes and coworkers found a somewhat linear increase of the prevalence with age in their Portuguese material.³ One may wonder if the higher prevalence in the older group, as well as a significantly higher prevalence in women also

TABLE 4. Occurrence of Occult Papillary Thyroid Carcinoma in a Japanese Study² Compared With Swedish Material

Age groups (yr)	No. of carcinoma/total glands	Prevalence
Hiroshima and Nagasaki, Japan*		
<24	†/41	4%
25-34	†/93	11%
35-44	†/154	24%
85-99	†/206	13%
Malmö, Sweden		
<24	3/29	10%
25-34	3/48	6%
35-44	5/84	6%
85-99	8/117	7%

* Two thirds of all glands sectioned at 2 to 3 mm intervals and visible lesions selected for microscopic examination. One fifth of the material less extensively examined. The rest sectioned at 2 to 3 mm intervals and each section examined microscopically.

† The exact number of carcinomas in each age group not given in the article.² It is stated without further comment that since the prevalence varied according to the category of examination (see above), data for the material as a whole had been standardized for this variable.

TABLE 5. Occurrence of Occult Papillary Thyroid Carcinoma in a Portuguese Study³ Compared With Swedish Material

Age groups (yr)	No. of carcinoma/total glands	Prevalence
Oporto, Portugal*		
10-19	0/39	—
20-29	0/38	—
30-39	3/64	5%
40-49	5/95	5%
70+	14/120	12%
Malmö, Sweden		
10-19	2/8	††
20-29	2/45	4%
30-39	4/62	6%
40-49	8/92	9%
>80	14/212	7%

* The glands sectioned at 3 mm intervals and visible lesions selected for microscopic examination.

†† The number of cases were too few to warrant a reliable estimation of the true frequency.

reported in this Portuguese study, is related to a generally larger size of the tumors making them easier to find in these groups. This phenomenon has been put forward by Sampson⁴ as being responsible for the female predominance of occult papillary thyroid carcinoma observed in the Japanese material from Hiroshima and Nagasaki. It is obvious that the results obtained in studies like these are highly dependent not only on diagnostic criteria and their subjective application, but also on the methods used because of the minute size of many of the lesions in question.

In the Swedish population studied by us, the frequency of occult papillary thyroid carcinoma does not seem to change very much with advancing age from the second decade. The situation in childhood is still unknown. It remains to be clarified whether most of these tumors arise early in life and then remain dormant with minimal growth, or whether new tumors steadily arise at all ages, some of which regress and vanish spontaneously, thus keeping the frequency at a relatively constant level. The comparatively few tumors that grow large enough to be recognized clinically and eliminated by surgery are of little importance in this context since the annual incidence of operated papillary thyroid carcinoma (including occult ones) is about 3/100,000 of the population in Malmö.

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Oxyphil Tumors of the Thyroid

Follow-up of 42 Surgical Cases

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Histopathologic and clinical follow-up data on 42 patients observed 2–20 years after operations for oxyphil neoplasms of the thyroid are presented. In eight patients histologic signs of malignancy were found but only two patients showed a clinically malignant course with development of distant metastases. The results do not indicate that oxyphil thyroid neoplasms are especially prone to assume a malignant course with the mode of treatment applied. Our policy is to remove any differentiated epithelial thyroid neoplasm with at least lobectomy. Total thyroidectomy is reserved for cases with capsular penetration, blood vessel invasion and/or metastases.

THE BEHAVIOR of neoplastic oxyphil tumors (Askanazy cell tumors, Hürthle cell tumors, oncocytomas) of the thyroid, and hence, the proper treatment of them is a matter of divided opinions. A report on this subject by Thompson and co-workers¹ indicates that all tumors of this type should be considered potentially malignant and treated with total thyroidectomy. In other studies, the prognosis has been considered to be favorable and simple lobectomy recommended as the treatment of choice.^{2,3} Regarding these contradictory statements and the very sparse literature in this field we think experience from a comparatively large material merits a report.

Materials and Methods

All patients in this study were living in the city of Malmö at the time of their thyroid operations. In this city, which has about 250 000 inhabitants, all thyroid surgery is performed at a single general hospital served by one department of pathology.

The histopathologic records of all surgical specimens registered as any kind of thyroid adenoma or carcinoma during a 19 year period (1960–1978) were reviewed. The microscopic slides were re-examined in all cases

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with descriptions indicating that an adenoma was composed of "large eosinophil and granular cells," "oxyphil cells," "Askanazy cells," "Hürthle cells," or "oncocytes." All cases coded as carcinomas were re-examined microscopically, irrespective of the cytologic description. Only lesions composed exclusively of oxyphil cells in available sections were selected for this study.

In cases in which the patient had concomitant multinodular goiter or chronic thyroiditis an oxyphil tumor was accepted as a neoplasm only if it had a distinct capsule.

Classification of a lesion as malignant was considered to require either blood vessel invasion, capsular penetration or metastases.

A total of 42 neoplastic oxyphil thyroid tumors were found. The patients could be traced in all cases. Thirty-five patients were alive. All of these went through a general physical examination with palpation of the neck. Furthermore serum thyroglobulin and alkaline phosphatases levels were determined as a screening for possible metastases. Chest x-rays were performed on 11 patients. Seven patients had died. Reports from complete autopsy examinations were available in all of these cases.

Results

The distributions by age at the time of operation and by sex of the 42 patients are given in Figure 1. The varying extent of the operative treatment is presented in Table 1. None of the patients had had any previous thyroid operation. The partial lobe resections were all performed in the early sixties. Since then, the surgical policy has been to remove a solitary nodule with at least complete lobectomy. The distribution of the patients

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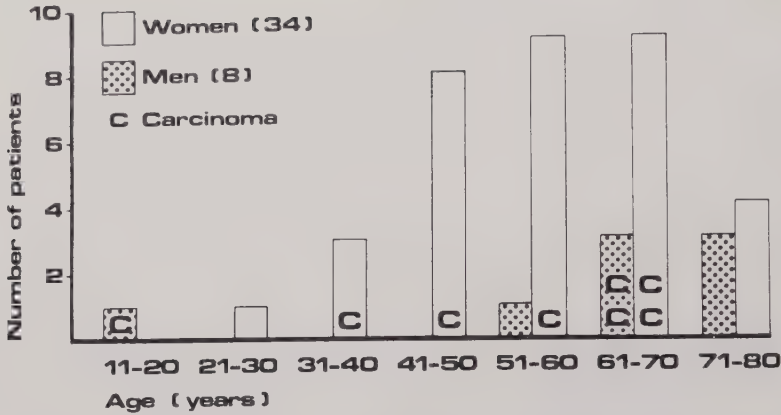


FIG. 1. Age and sex of 42 patients with oxyphil thyroid tumors.

according to the size of the tumors is given in Table 2. No tumor was less than 1 cm in largest dimension.

Three oxyphil tumors occurred in multinodular goiters and one was associated with chronic thyroiditis. Furthermore, there was a toxic diffuse goiter in one patient, and in two patients each an autonomous follicular adenoma was present in addition to the oxyphil tumor. The oxyphil tumors were solitary in all but two cases. One patient had three separate well encapsulated oxyphil tumors, all present in the isthmus region and measuring 1 cm each. Another patient had two operations for oxyphil tumors, one in each lobe, with an interval of one year. Both tumors were examined extensively without disclosing any histologic signs of malignancy. Thus, the second tumor was considered to be an additional adenoma and not a metastasis from the first tumor.

In eight of the 42 oxyphil tumors, histologic signs of malignancy were found. However, only two of these cases have, as yet, shown a clinically malignant course with development of metastases. One of these—a 69-year-old woman at the time of operation—developed lung metastases after eight years. The other patient was

a 63-year-old man who had lymph node metastases in the neck at the time of operation and developed liver metastases two years later. In both cases, which had been treated with total thyroidectomy, the distant metastases accumulated radioactive iodine. The serum thyroglobulin level was increased to five times the upper limit of normal in both patients at follow-up examination.

None of the remaining 33 living patients showed any signs of local recurrence or metastases. The observation time for these patients is given in Figure 2. All had been followed for at least two years.

None of the seven dead patients showed any local disease recurrence or metastases of their oxyphil thyroid tumors at autopsy examination. In one of these patients, the tumor was considered to be malignant because of capsular penetration.

Discussion

This study does not indicate that oxyphil neoplasms of the thyroid are especially prone to assume a clinically malignant course, and the results contrast markedly to the dismal prognosis found in the study of Thompson and co-workers.¹ Eleven of their 25 patients with oxyphil thyroid neoplasms were reported to have died from their tumors, while another four patients, who were still

TABLE 1. Operations Performed in 42 Patients with Oxyphil Thyroid Tumors

Type of Operation	All Cases	Number of Carcinomas
Partial lobe resection*	9	—
Hemithyroidectomy†	27	4
Total thyroidectomy	6	4

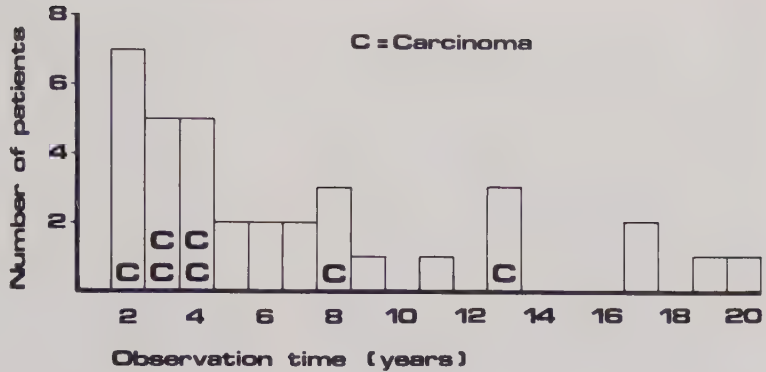
* Including 2 bilateral subtotal thyroidectomies.

† Including 4 cases of lobectomy on the tumorbearing side + partial resection of the other lobe.

TABLE 2. Size of 42 Oxyphil Thyroid Tumors (Largest Dimension)

Tumor Size (cm)	All Cases	Number of Carcinomas
<2	14	2
2-4	20	5
>4	8	1

FIG. 2. Observation time of 35 patients alive at follow-up.



alive, had developed local recurrences or distant metastases.

The cause of this discrepancy is probably, at least in part, to be searched for in the composition of the materials and methods used to collect them. As for the composition, Thompson and co-workers state that many of their patients were referred to them after initial treatment at other institutions. Thus, it is reasonable to presume that prognostically bad cases were accumulated in their material. As for the methods used to collect the material, Thompson and co-workers reviewed only those specimens that had been coded as oxyphil thyroid tumors. In that way, tumors of this type not specifically coded as such escape detection, which may influence the figure for the relative frequency of malignancy in the material.

Another crucial point is the follow-up of the deaths in the material. It does not appear from the article by Thompson and co-workers to what extent autopsy examinations had verified the source of metastases in those patients who were reported to have died from spread of their oxyphil tumors. Four of the seven deaths in our own material were caused by metastases, which, however, were shown to originate from neoplasms other than the oxyphil thyroid tumors.

Differences regarding the frequency of histopathologically malignant cases may be ascribed to variations in the extent of tissue sampling for microscopic examination, in the diagnostic criteria used and the subjective application of these criteria. However, the frequency of histopathologic malignancy is not very interesting *per se*, but more important is the frequency of clinical malignancy.

Information, or rather lack of information, regarding the methods used to collect the materials in the few larger series of oxyphil thyroid tumors published besides

the one of Thompson and co-workers⁴⁻¹⁰ lend little value to these series as indicators of the relative frequency of clinical malignancy among this type of tumor. However, we think the methods used in the present study permit our findings to shed light on this issue, at least as concerns the region of Malmö, since they reflect the conditions in a demographically as well as clinicopathologically well defined material.

A factor which may influence the relative frequency of clinical malignancy is, besides the type of treatment, at what stage of disease the treatment is instituted. However, it is very difficult, if not impossible, to make comparisons in this respect. To exclude the possibility that a falsely good prognosis in our material is related to absence of cases never operated because of too advanced disease at the time of admission, we have checked the autopsy reports of patients who died of thyroid carcinoma. During the period 1960-1978, 32,539 autopsy examinations were performed at the Department of Pathology, which corresponds to 95% of all patients who had died in hospital in the city of Malmö. The autopsy examinations were performed in a standardized way, including examination of the thyroid gland at least macroscopically. In 33 patients thyroid carcinoma was registered as the principal cause of death. In only two of these, the carcinomas were of pure oxyphil cell type. Both had been considered inoperable and given radiotherapy only.

Since metastases of oxyphil thyroid carcinomas occasionally develop more than a decade after the initial treatment, it may be objected that the observation time is short in part of the present material. Nevertheless, these results cast doubt upon the validity of the conclusion of Thompson and co-workers that all oxyphil neoplasms of the thyroid should be considered as potentially malignant and treated aggressively with total

thyroidectomy irrespective of the histopathologic picture.

From the present experience we find no reason to treat neoplastic oxyphil thyroid tumors otherwise than other differentiated epithelial thyroid neoplasms. If there are no obvious signs of malignancy at operation, such as infiltration of surrounding tissues or metastases, we perform a lobectomy and await the diagnosis from microscopic examination of permanent sections. If histologic signs of malignancy are in evidence a second operation for total thyroidectomy is performed.

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Morphometric Studies on Nuclei in Smears of Fine Needle Aspirates from Oxyphilic Tumors of the Thyroid

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Morphometry was applied to smears of fine needle aspirates from 26 oxyphilic thyroid neoplasms. Thirteen tumors were considered benign as judged from histologic findings and clinical follow-up for 2 to 20 years after the operation. Thirteen tumors were considered malignant histologically; four of these had proven metastases. In each case the mean and standard deviation of the projected nuclear area were calculated from 200 nuclei in the smear. Our results indicate that morphometric estimation of mean nuclear size and/or degree of anisokaryosis is of no practical value in distinguishing between benign and malignant thyroid neoplasms of the oxyphilic type.

With regard to thyroid neoplasms of the oxyphilic type (Hürthle-cell tumors, Askanazy-cell tumors, oncocytomas), opinion is divided as to whether the cytologic appearance permits a differentiation between benign and malignant growths. Some investigators^{4,5} do not believe this distinction can be made, while others^{6,7} consider it possible. It is difficult to evaluate these contradictory views because they are based on subjective interpretations. The purpose of this study was to assess objectively two cytologic features, i.e., the mean nuclear size and the degree of anisokaryosis, both of which have been claimed to be important to this differentiation, and hence to evaluate the usefulness of morphometry as a diagnostic tool in this context.

Materials and Methods

Smears of fine needle aspirates from 26 oxyphilic thyroid neoplasms were used for this study. All smears were air dried and stained according to May-Grünwald-Giemsa. Histologic sections were available in all cases. Only tumors composed exclusively of oxyphilic cells in the examined sections were included in this study. Thirteen tumors were classified as malignant; nine of these showed capsular penetration and/or blood vessel invasion as the only histologic signs of malignancy, whereas the remaining four in addition had histologically verified metastases. Thirteen tumors were interpreted as benign on the basis of the histologic picture as well as the clinical follow-up, which has been described in detail elsewhere.¹ The observation periods of these 13 patients varied from 2 to 20 years, with a median of 6.

A Leitz ASM image analysis system and a Leitz orthoplan microscope (E. Leitz, Wetzlar, West Germany) were used for the morphometric studies. In each one of the cytologic smears, the individual projected nuclear areas of 200 tumor cells were measured. The perimeter of each nuclear profile was measured using the drawing pen of the image analysis system, and the profile area was calculated by the built-in computer, which also calculated the mean

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Table I Morphometric Results Obtained from Fine Needle Aspirates of 26 Oxyphilic Thyroid Neoplasms

Case no.	Projected nuclear area		
	Mean (sq μm)	S.D. (sq μm)	C.V. (%)
<i>Tumors considered benign histologically and clinically</i>			
1	71	21	30
2	72	20	28
3	82	36	44
4	89	32	36
5	90	20	22
6	92	18	20
7	92	24	26
8	92	29	32
9	94	15	16
10	95	33	35
11	118	34	29
12	121	52	43
13	142	61	43
<i>Tumors considered malignant histologically and without proven metastases</i>			
1	75	16	21
2	76	34	45
3	78	18	23
4	106	30	28
5	114	38	33
6	123	49	40
7	133	24	18
8	141	40	28
9	149	33	22
<i>Malignant tumors with histologically verified metastases</i>			
1	98	24	24
2	100	17	17
3	107	49	46
4	118	80	68

S.D. = standard deviation, C.V. = coefficient of variation. Individual means were calculated from 200 nuclei in each case.

and standard deviation of the projected nuclear area for the 200 nuclei per smear. Each smear was first surveyed under low magnification to select and demarcate areas with well-preserved tumor cells judged to be representative of the material as a whole. The selected areas were systematically scrutinized by use of a 100 $\times$ oil immersion objective. The magnification on the measuring board was about 1,400 $\times$. In each field of view, all intact tumor cell nuclei were measured, with the exception of naked nuclei. The reason why naked nuclei were excluded was to avoid measurement of nuclei from irrelevant cells, such as histiocytes and lymphocytes, which occurred in great numbers in some aspirates because of degenerative changes or lymphoid infiltration of the tumors. The measurements were performed by one of the authors without knowledge of the histopathologic diagnoses.

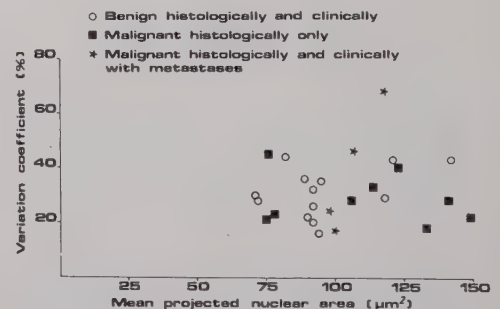
Results

The values for mean and standard deviation of the

projected nuclear area in each case are listed in Table I. The mean of the projected area is plotted against the coefficient of variation in Figure 1 to illustrate the distribution of these two variables in relation to the presence or absence of histopathologic and clinical signs of malignancy.

The reproducibility of the results was checked by letting the same observer perform a second measurement of the five most anisokaryotic specimens. For the mean projected nuclear area, the second value differed by 9% from the first in one case (with the highest variation coefficient) and by less than 5% in the others. The coefficients of variation differed by at most one-tenth of the first value. Furthermore, the variation from smear to smear depending on technical factors involved with the smear preparation was tested by measuring the mean projected nuclear area of 25 lymphocytes in each of four specimens. The two malignant cases with the smallest and the two benign cases with the largest mean tumor-cell nuclear size were chosen for this comparison. The largest lymphocytic mean nuclear size differed by 5% from the smallest one.

The consequence of excluding all naked nuclei from measurement was tested by a second examination of some specimens performed the same way as the first one except for including naked nuclei in the measurements. The four malignant specimens (two cases with and two without metastases) showing the smallest mean nuclear size and the least-pronounced anisokaryosis were chosen for this test since they were crucial to the conclusion drawn from the whole study. Furthermore, these smears were well suited to the test since they showed virtually pure tumor-cell

**Figure 1**

Distribution of nuclear size (mean projected nuclear area) and degree of anisokaryosis (coefficient of variation) in relation to the presence or absence of histologic and clinical signs of malignancy in 26 oxyphilic thyroid neoplasms.

aspirates without any noteworthy admixture of irrelevant cells, such as lymphocytes and histiocytes. For the mean projected nuclear area, the second value differed at most by 6% from the first one (92 sq μm and 98 sq μm , respectively), and the difference was only 1% in two cases. As regards the difference between coefficients of variation, this was limited to a single percentage step in all four cases.

Discussion

Morphometric estimation of the mean nuclear size of tumor cells in smears of fine needle aspirates from follicular neoplasms of the thyroid has been reported to permit differentiation between benign and malignant tumors with a high degree of probability.^{2,3} In the studies referred to, however, tumors of the oxyphilic type were excluded, and we have not been able to find any other published studies in which morphometry was applied to this kind of tumor.

In the cytologic material from oxyphilic thyroid neoplasms presented here, the malignant tumors were found to have a somewhat larger mean nuclear size than the benign ones when compared as groups. There was, however, an almost complete overlapping between the two tumor categories with regard to individual values. The degree of anisokaryosis, expressed as the coefficient of variation, likewise showed an almost complete overlapping between benign and malignant tumors.

Some of the overlapping between benign and malignant tumors in material of this kind might be explained by technical factors involved with the smear preparation, which can never be completely standardized. It should be noted, however, that the type of preparation used in this study is simple to perform, which is favorable as regards the comparability of the specimens, and a test of the variation from smear to smear using the lymphocyte size for comparison showed only minor differences. Furthermore, one person performed the series of measurements presented here, which ought to have minimized methodologic variation with regard to the selection of what was to be measured. Ideally, all intact tumor-cell nuclei in the smears should be measured to avoid a sampling error. Even if measurement of all intact nuclei were planned, however, the problem of subjectivity in the interpretation of which nuclei are intact would remain. Furthermore, the smear itself is a minute point sample of the whole tumor-cell mass, which is not always homogenous. These factors cannot be fully eliminated, and if the method in question is to be of any practical value as guidance in the

choice of treatment, the difference between benign and malignant lesions must be large enough not to be obscured by such factors. The reproducibility of the results obtained by the methods used here is, in our opinion, sufficient for the purpose of this study. The exclusion from measurement of all naked nuclei irrespective of size did not influence the results significantly. Accompanying cytoplasm of oxyphilic cells, whether intact or not, was used as a marker of which nuclei to measure. This fact was practically important since it allowed a reliable examination of tumor aspirates with a prominent admixture of histiocytes or lymphocytes.

It may be objected that cases with marked anisokaryosis and large mean nuclear size, considered benign in this study, could in fact have been carcinomas despite the lack of histologic signs of malignancy in the tissue samples examined. This possibility cannot be completely excluded even by our thorough clinical follow-up since, on the one hand, a carcinoma may of course have been treated successfully and, on the other hand, metastases of oxyphilic thyroid carcinomas may develop more than a decade after the initial treatment. Even if a few diagnostic errors theoretically may have contributed to the overlapping between benign and malignant tumors in our material, the most important finding would be that several carcinomas occurred among the tumors with the smallest mean nuclear size as well as the least-pronounced anisokaryosis. Thus, malignancy cannot be ruled out by this method.

Our results indicate that morphometric estimation of nuclear size and/or degree of anisokaryosis is of no practical value in distinguishing between benign and malignant thyroid neoplasms of the oxyphilic type.

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NUCLEAR DNA CONTENT AND BEHAVIOUR OF OXYPHIL THYROID TUMORS

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ABSTRACT

Microspectrophotometric measurement of nuclear DNA content was made on archival smears of fine needle aspirates from 23 oxyphil thyroid neoplasms. Fourteen tumors were considered benign as judged from the histologic picture as well as follow-up for seven to 18 years after the operation. Nine tumors were malignant; five of these showed capsular penetration and/or blood vessel invasion as the only signs of malignancy, whereas the remaining four in addition had histologically verified metastases and were the cause of death. The DNA patterns found (diploid, polyploid or aneuploid) appeared to have a limited diagnostic value since malignancy could not be excluded on this basis. A practically useful finding was, however, that aneuploidy appeared to be associated with a high probability of invasive growth. As regards prognostic information, it was found that euploid patterns occurred in tumors from patients with long survival after surgical treatment, while tumors with aneuploid patterns showed a variable clinical course.

INTRODUCTION

Opinion is divided as to whether it is possible to make a cytologic distinction between benign and malignant oxyphil tumors of the thyroid (also called Hürthle cell tumors, Askanazy cell tumors and oncocytomas). The contradictory views of different investigators are difficult to evaluate since they have been based on subjective interpretations. Morphometry was recently used to objectively assess two cytologic features - the mean nuclear size and the degree of anisokaryosis - which have been claimed to be important to this differentiation. However, neither of these features was found to be of any practical value (1). In the present study another cytologic element - the nuclear DNA content - has been quantified objectively to evaluate its bearing on the behaviour of oxyphil neoplasms of the thyroid.

MATERIAL AND METHODS

The study is based on archival smears of fine needle aspirates from 23 oxyphil thyroid neoplasms, which have been subjected to a previously reported clinicopathologic investigation (2). Nine tumors were classified as malignant; five of these showed capsular penetration and/or blood vessel invasion as the only signs of malignancy, whereas the remaining four in addition had histologically verified metastases and were the cause of death. Fourteen tumors were interpreted as benign on the basis of the histologic picture as well as the clinical course. At present the follow-up ranges from seven to 18 years, and so far none of the patients has presented any local recurrence or metastases of the tumor in question.

All smears were originally air-dried and stained according to May-Grünwald-Giemsa. After destaining in methanol, the smears were fixed in 10% buffered neutral formalin for a minimum of 15 hours and Feulgen stained. Acid hydrolysis was performed in 5N HCl at 22°C for 60 minutes. DNA measurements were made in a rapid scanning and integrating microspectrophotometer at 546 nm (3). One hundred tumor cell nuclei were measured in each case. Leukocytes were used as internal normal controls. For comparison of the DNA pattern in different tumors, the nuclear DNA content was expressed in c-units with 2c representing the mean value of the normal diploid control cells in the individual specimen. Measurement and classification of the DNA pattern was made without knowledge of histopathologic diagnosis or clinical course.

RESULTS

The nuclear DNA patterns of the tumors examined could be divided into three main categories (Figure 1). Firstly, diploid tumors characterized by DNA values in the same region as the normal control cells (2c), and secondly, polyploid tumors with cell populations in the tetraploid (4c) and sometimes octaploid (8c) regions in addition to a diploid population. Cases with a minority population of cells showing DNA values between the 2c and 4c regions were included in this category. The third category comprised aneuploid tumors with DNA patterns deviating from those mentioned. In Figure 2 the different DNA patterns have been correlated to the histologic picture of the tumors and their clinical course.

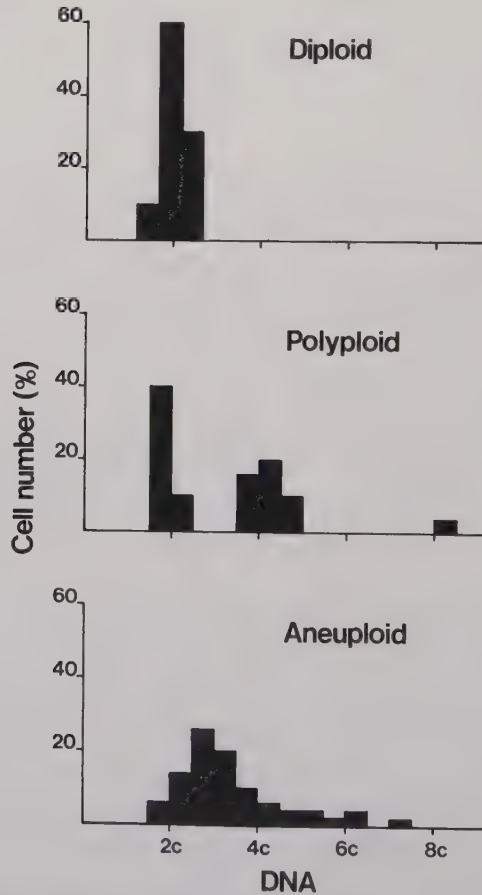


Figure 1. Representative examples of the three main patterns of nuclear DNA content in the oxyphil thyroid tumors examined. The DNA values are expressed in c-units with 2c representing the mean value of the normal diploid control cells in the individual specimen.

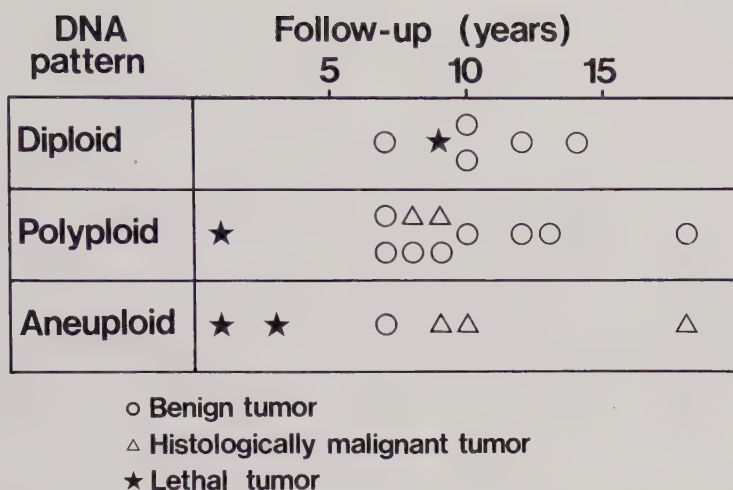


Figure 2. The main DNA patterns in 23 oxyphil thyroid neoplasms correlated to their histologic picture and clinical course. For lethal tumors the time from diagnosis to death of the patient is given. The two patients with shortest survival had advanced disease with inoperable primary tumors and distant metastases already when first examined. None of the living patients had presented any evidence of local recurrence or metastases at the time of this study.

DISCUSSION

Some investigators have claimed that determination of nuclear DNA content in fine needle aspirates may be a useful tool for preoperative differentiation of benign from malignant thyroid lesions (4). However, the study in question comprises a relatively small number of cases in which routine cytology usually does not permit a conclusive diagnosis with regard to malignancy, i.e. follicular tumors. Other investigators (5,6) consider the diagnostic value of DNA measurements to be very limited in this context, since carcinomas may show patterns indistinguishable from those in adenomas and non-neoplastic lesions such as colloid goiter and chronic thyroiditis. On the other hand, it has been claimed that DNA measurements offer useful information additive to conventional microscopic evaluation concerning the prognosis in follicular tumors and differentiated carcinomas of the thyroid (6-8).

As regards oxyphil tumors of the thyroid, we have not been able to find any other published study correlating the DNA patterns of this particular type of neoplasia with long-term follow-up of the patients. Our results indicate that the method used has a limited value for achievement of a preoperative diagnosis with regard to tumor behaviour. Most important is that malignancy cannot be ruled out, which is in agreement with the situation reported for non-oxyphil follicular neoplasms of the thyroid (5,6), as well as neoplasms in other tissues like for example the mammary gland (9). Even diploid tumors showing normal nuclear DNA amounts may occasionally metastasize and kill the patient (Figure 3). In this context it should be observed that the finding of DNA amounts within normal limits does not exclude chromosomal abnormalities. It may merely be a reflection of DNA changes that are too small to be measurable with the method used (10).

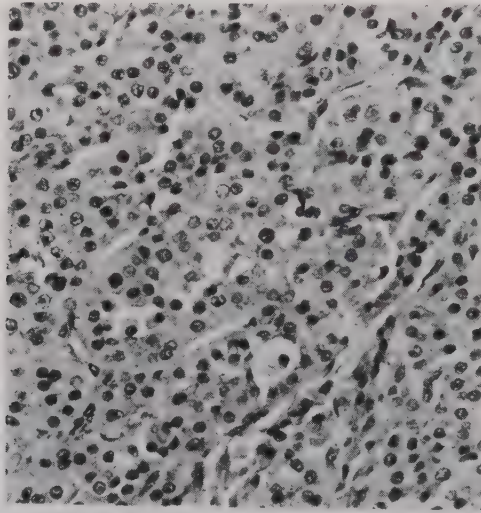


Figure 3. Diploid oxyphil thyroid carcinoma which metastasized and killed the patient. Note the uniform and bland nuclei. Malignancy cannot be ruled out by means of cytology or DNA analysis in this type of tumor (H&E, X330).

A useful fact is that we found aneuploid DNA patterns to be associated with histologic signs of malignancy in five out of six cases. This finding is in line with the opinion of others (4,6-8) that aneuploidy reflects a high

potential of malignancy in thyroid tumors, and our single exception without proven invasive growth may be explained by insufficient tissue sampling for histologic examination since very few sections from the capsular region were available in that case. In another series of thyroid lesions (4), four aneuploid tumors specified to be of oxyphil type all were malignant. Demonstration of tumor aneuploidy by means of fine needle aspiration may thus be of practical value as a decisive reason for operation, especially in surgical high risk patients (Figure 4). It shall be stressed that - although frequently associated with invasive growth - aneuploidy did not indicate a generally poor prognosis with regard to patient survival in this material. Four of the six patients in question are still alive seven to 18 years after surgical treatment, and so far none of them has presented any evidence of local recurrence or metastases.

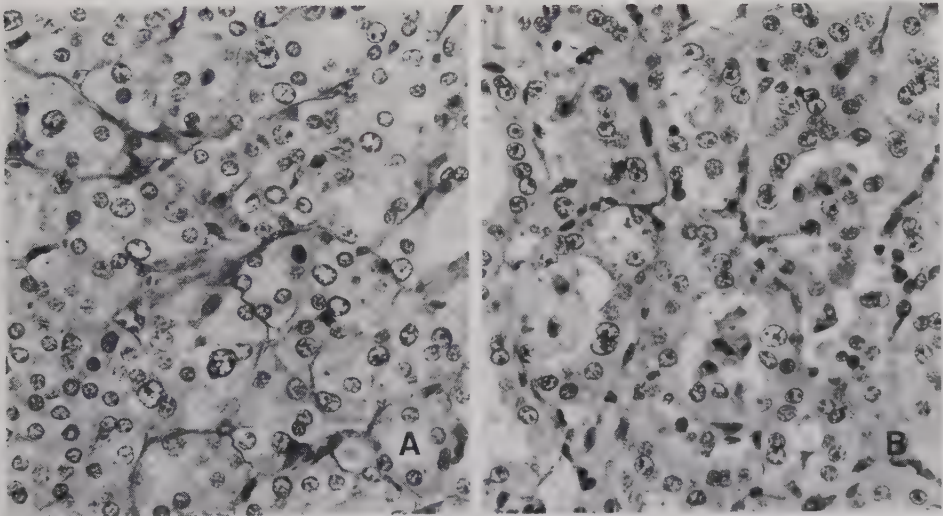


Figure 4. Polyploidy in a benign oxyphil thyroid tumor (A) may give aniso-karyosis of the same degree as aneuploidy in a malignant one (B), and cytologic diagnosis of malignancy is usually not possible in this type of tumor. Although DNA analysis cannot exclude malignancy, a practically useful fact is that high risk cases with regard to invasive growth can be identified by demonstration of aneuploidy (H&E, X330).

In the polyploid group we encountered two benign and two malignant tumors that showed comparatively numerous cells with DNA values between peaks at 2c and 4c. This finding is consistent with an increased proliferative activity compared to other specimens in this group, as reflected by more numerous cells in what corresponds to the DNA synthesis phase region of cells with normal DNA value. The same picture has been observed in non-neoplastic goiters (4,5) which means that it does not give any practically useful diagnostic information.

In conclusion, our results indicate - with reservation for the relatively small number of cases examined so far - that DNA analysis with the method used here has a limited diagnostic value in oxyphil thyroid tumors. Aneuploidy seems to be associated with a high probability of invasive growth, but no DNA pattern permits exclusion of malignancy. As regards prognostic information, it was found that euploid patterns occurred in tumors from patients with long survival after surgical treatment, while tumors with aneuploid patterns showed a variable clinical course.

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